IN THE SPECIFICATION

Page 6, insert the following heading and paragraph starting on line 16:

Brief Description of The Drawing

Figure 1 shows induction of antigen-specific T cells by immunisation of mice with *Lactobacillus plantarum* pLP503 tetanus toxin fragment C (TTFC). Mice were immunised intranasally or orally with wild type *L. plantarum* 256 or transformed *L. plantarum* pLP503-TTFC followed by an identical booster immunisation on days 28-30. Spleen cells and cervical lymph node (CLN) cells were harvested and stimulation following exposure to TTFC, TT, TT peptide, P30 or medium alone was measure by [^{3}H] thymidine incorporation. Each set of bars represents, from left to right, the results for oral immunisation with *L. plantarum* pLP503-TTFC, oral immunisation with wild type *L. plantarum* 256, intranasal immunisation with *L. plantarum* pLP503-TTFC and intranasal immunisation with *L. plantarum* 256. Figs. 1A and 1C show the results 12 days after the final boost for spleen and CLN cells respectively. Figs. 1B and 1D show the results 21 days after the final boost for spleen and CLN cells respectively. Results are expressed as the stimulation index (SI) calculated for the mean counts per minute (c.p.m.) of triplicate test cultures of cells divided by the mean c.p.m. of cultures receiving buffer alone.

Page 28, replace the third paragraph starting on line 17, with the following:

Proteins in 30μg of total cell extracts or fractions were separated by SDS-polyacrylamide gel electrophoresis (PAGE), (10% acrylamide, 400mM Tris [pH 8.9]) and run in a 25mM Tris, 192mM glycine buffer (pH 8.3) at 200V for 45 mins. Protein was transferred electrophoretically onto nitrocellulose using a Biorad™ electrophoresis unit. Immunoblots were developed using optimally diluted rabbit TTFC-specific antiserum and goat-anti-rabbit IgG-specific phosphatase conjugates (Nordic™, Tilburg, The Netherlands).

Page 28, replace the fourth paragraph starting on line 26, with the following:

At defined time points bacteria were prepared for analysis by FACScan™. Cells were washed twice and re-suspended in PBS/1% Bovine serum albumin (BSA). 50µl of optimally diluted Rabbit TTFC-specific antiserum was added to the cells for 1 hour. Cells were again washed twice and bound antibody was detected by a 30 min incubation with fluorescein isothiocyanate-conjugated (FITC) anti-rabbit at a dilution of 1:1000. Cells were then washed twice prior to analysis for light scatter and fluorescence on a Becton-Dickinson™ flow cytometer. A gate was set around appropriate size events as determined by cytograms of forward and side scatter. Controls were prepared by staining wild-type *L. casei* 393 or *L. plantarum* 256, staining recombinants using non-immune rabbit serum or excluding the rabbit TTFC-specific antiserum. All procedures were performed on ice with 1% BSA. For each sample data was collected for 10,000 - 20,000 gated events. The fluorescence obtained from bacterial cell suspensions was represented by fluorescence histogram and mean channel intensities calculated.

Page 29, replace the fifth paragraph starting on line 31, with the following:

Antigen-specific immunoglobulin G (IgG) levels were evaluated using microtiter plates coated o/n with 50µl of a 0.16µg/ml solution of TT (RIVM™, Bilthoven, The Netherlands). Individual serum samples were titrated by serial $\log_2$ dilutions and assayed in duplicate. Bound antibody was detected by the addition of 50µl of optimally diluted goat anti-mouse IgG-phosphatase conjugate (Nordic™, Tilburg, The Netherlands). Following the addition of the PNPP (1mg/ml in 0.1M DEA/ $MgCl_2$) chromogen substrate, antibody levels were quantified by measuring plate A405nm values obtained 30-90 minutes following the initiation of reaction. End-point titres were calculated using a cut-off determined from the mean absorbance (OD 0.2) of a 1:10 dilution of serum obtained from pre-immune mice. For antigen-specific immunoglobulin A (IgA) levels in broncho-alveolar lavage fluid the same procedure was performed, using an optimally diluted goat anti-mouse IgA phosphatase conjugate (Nordic™).

Page 30, replace the first paragraph starting on line 13, with the following:

At 12 and 21 days following the last immunisation, the spleens and cervical lymph nodes (CLN) of mice were removed aseptically. Single cell suspensions were prepared by passage through a cell strainer (70uM Nylon; B&D™ [[,]]), and centrifugation at 1500rpm for 10 min. Viable, un-fractionated cell numbers were assessed by Trypan blue dye exclusion.

Page 30, replace the second paragraph starting on line 18, with the following:

For testing antigen-specific T-lymphocyte proliferation, cells were resuspended and plated at concentrations of $3x10^5$ cells/spleen or $5x10^5$ cells/LN in a final volume of 200µl culture medium (RPMI-1640, supplemented with 10% heat-inactivated fetal calf serum, 2 mM-L-glutamine, 20U/ml penicillin and 20 µg/ml streptomycine (all Gibco™, Pairsley, UK), and 50µM 2-mercaptoethanol {Sigma™, MO}) in sterile flat-bottomed 96-well culture plates (Nunc™, Denmark). Control wells contained medium only, and antigens were added to triplicate cultures over the indicated dose range. All cells were maintained in a humidified 5% CO_2 atmosphere at 37°C for 4 days. The cells were pulse-labelled with 0.6uCi[^{3}H] thymidine (^{3}H-TdR, 5 Ci/mMol, TRA 120, Amersham™, UK) in 30 µl volumes/culture well during the last 16-18 hours before harvesting. Cells were collected using an ILACON™ cell harvester and deposited onto glass fibre filter discs (Whatman™). Thymidine incorporation was assessed by gas scintillation spectrometry (β-plate counter, Canberra Packard™, Meriden, CT) and the results were calculated as the mean c.p.m (± SD) from triplicate cultures and expressed as a stimulation index (SI).

Page 31, replace the first paragraph starting on line 1, with the following:

Quantifying the number of TT-specific antibody-secreting cells (ASC) in the spleen and CLN was undertaken according to Czerkinsky *et al*,J. Immunol. Methods 65: 109 (1983). Microtiter plates (Maxisorp™ plates, Nunc, Denmark) were coated o/n with 50µl of a 0.16µg/ml solution of TT (RIVM™, Bilthoven, The Netherlands) or 50µl of PBS as control. After extensive washing, and blocking of the plate with PBS/0.1%BSA, cells were added at concentrations of $1x10^6$ and $2x10^5$ cells/spleen or $5x10^5$ and $1x10^5$ cells/LN into

a final volume of 50µl culture medium and incubated for 4h in a humidified 5% CO_2 atmosphere at 37°C. The plates were rinsed and incubated for 20 min. with ice-cold PBS containing 10mM EDTA to remove the cells, and washed again with PBS/0.05% Tween-20™, and PBS/0.5% BSA. Bound antibody was detected by the addition of 50µl of optimally diluted rabbit anti-mouse Ig phosphatase-conjugate (DAKO™, Denmark) o/n at 4°C. Plates were washed extensively, and incubated with 1mg/ml BCIP in AMP buffer containing 1% low melting temperature agarose. The plates were inverted over a light source, and macroscopically blue dots were scored.

Page 31, replace the third paragraph starting on line 22, with the following:

Specifically, pLP401-TTFC transformants (surface-anchored expression) were grown in LCM (+2% mannitol) and pLP503-TTFC transformants (intracellular expression) in MRS (Difco™), (both supplemented with 5µg/ml erythromycin), at 37°C to an OD 0.6, pelleted and disrupted by sonification. 30µg total protein was analysed on a 10% SDS/polyacrylamide gel and separated proteins transferred to nitrocellulose electrophoretically. TTFC was visualised with rabbit anti-TTFC (1:500) and a phosphatase/PNPP chromogen combination. Bars indicate the migration of molecular weight markers.

Page 31, replace the fourth paragraph starting on line 30, with the following:

Immuno-fluorescence analysis was performed of recombinant *L. plantarum* pLP401-TTFC and *L. casei* pLP401-TTFC expressing TTFC as a surface-anchored product. Lactobacilli were gated on the basis of forward and side scatter and stained with rabbit TTFC-specific antiserum diluted 1:500 (Calbiochem™, Ca). Bound antibody was detected with optimally diluted FITC-conjugated anti-rabbit IgG (Jackson™, PA). Fluorescence levels from cells collected at OD 0.6 were analysed by FACScan™ (Becton Dickinson™) and shown in histogram form, presented in relation to levels of fluorescence obtained with non-recombinant lactobacilli. 10,000 cells were analysed in each experiment.

Page 32, replace the first paragraph starting on line 7, with the following:

From the photograph of the protein expression and the Mackintosh™ generated FACscan™ it was shown that lactobacilli containing pLP503-TTFC express only the intracellular 50kDa TTFC polypeptide. *L. plantarum* containing the vector pLP401-TTFC express a surface-anchored 75kDa polypeptide corresponding to the 50kDa TTFC fused to an anchor sequence of 25kDa, at a level higher than for *L. casei* pLP401-TTFC. Exposition of TTFC on the cell-wall of *L. plantarum* and *L. casei* through fusion to the anchor sequence was confirmed by FACscan™.